

THAT WHICH IS CLAIMED IS:

- Sub B1*
1. A recombinant adeno-associated virus (rAAV) vector comprising a heterologous nucleotide sequence encoding B-domain deleted factor VIII operably linked with at least one enhancer and at least one promoter.
 2. The rAAV vector of claim 1, wherein said rAAV vector further comprises spacer DNA.
 3. The rAAV vector of claim 1, wherein said rAAV is selected from the group consisting of AAV serotype 1, serotype 2, serotype 3, serotype 4, and serotype 5.
 4. The rAAV vector of claim 1, wherein said B-domain deleted factor VIII is a human B-domain deleted factor VIII.
 5. The rAAV vector of claim 4, wherein said heterologous nucleotide sequence encodes a B-domain deleted factor VIII having the amino acid sequence set forth in SEQ ID NO:2.
 6. The rAAV vector of claim 4, wherein said heterologous nucleotide sequence comprises the sequence given as about nucleotides 419 to 4835 of the nucleotide sequence set forth in SEQ ID NO:1.
 7. The rAAV vector of claim 1, wherein said promoter is an AAV ITR.
 8. A pharmaceutical formulation comprising the rAAV vector of claim 1 in a pharmaceutically acceptable carrier.

9. A recombinant adeno-associated virus (rAAV) vector comprising a heterologous nucleotide sequence encoding factor VIII operably linked with a liver-preferred expression control element.

10. The rAAV vector of claim 9, wherein said heterologous nucleotide sequence comprises the sequence given as about nucleotides 419 to 4835 of the nucleotide sequence set forth in SEQ ID NO:1.

11. The rAAV vector of claim 9, wherein said liver-preferred expression control element comprises at least one enhancer selected from the group consisting of the α 1 microglobulin/bikunin enhancer, the hepatitis B virus EnhI enhancer, the hepatitis B virus EnhII enhancer, the human albumin E_{1,7} enhancer, and the human albumin E₆ enhancer.

12. The rAAV vector of claim 9, wherein said liver-preferred expression control element comprises the hepatitis B virus EnhI enhancer given as about nucleotides 419 to 4835 of the nucleotide sequence set forth in SEQ ID NO:1.

13. The rAAV vector of claim 9, wherein said liver-preferred expression control element comprises at least one promoter selected from the group consisting of the hepatitis B virus core promoter, the mouse albumin promoter, the human U1 snRNA promoter, and the herpes simplex virus thymidine kinase promoter.

14. The rAAV vector of claim 9, wherein said liver-preferred expression control element comprises at least one transcription factor binding site selected from the group consisting of a TATA box, a CAAT box, a GC box, an ATF box, a C/EBP binding site, an HNF1 binding site, an HNF2 binding site, an HNF3 binding site, an HNF4 binding site, and a TGT3 binding site.

15. The rAAV vector of claim 9, wherein said heterologous nucleotide sequence further comprises sequences encoding a promoter and a polyadenylation sequence.

5 16. The rAAV vector of claim 9, wherein said heterologous nucleotide sequence comprises the sequence given as about nucleotides 150 to 4914 of the nucleotide sequence set forth in SEQ ID NO:1.

10 17. The rAAV vector of claim 9, wherein said heterologous nucleotide sequence encodes the amino acid sequence set forth in SEQ ID NO:2.

15 *Sub a* → 18. A recombinant adeno-associated virus (rAAV) vector comprising a heterologous nucleotide sequence encoding a B-domain deleted factor VIII operably linked with an enhancer, wherein said nucleotide sequence is selected from the group consisting of:

- 20 (a) the nucleotide sequence given as nucleotides 419 to 4835 of the nucleotide sequence set forth in SEQ ID NO:1,
- (b) a nucleotide sequence that hybridizes to the nucleotide sequence of (a) under conditions of high stringency and which encodes a B-domain deleted factor VIII, and
- (c) a nucleotide sequence that that differs from the nucleotide sequences of (a) and (b) above due to the degeneracy of the genetic code, and which encodes a B-domain deleted factor VIII.

25 19. The rAAV vector of claim 18, wherein said rAAV further comprises spacer DNA.

30 20. A composition comprising a population of at least about 10^{12} recombinant adeno-associated virus (rAAV) vector particles comprising a heterologous nucleotide sequence encoding B-domain deleted factor VIII.

21. A method of delivering a nucleotide sequence encoding B domain-deleted factor VIII to a cell comprising contacting the cell with a recombinant adeno-associated virus (rAAV) vector comprising a heterologous nucleotide sequence encoding B-domain deleted factor VIII operably linked with a liver-preferred expression control element.

22. The method of claim 21, wherein the contacting is carried out *in vitro*.

23. The method of claim 21, wherein the contacting is carried out *in vivo*.

24. The method of claim 21, wherein the cell is selected from the group consisting of neural cells, liver cells, muscle cells, retinal cells, epithelial cells, fibroblast cells, germ cells, bone marrow cells, hematopoietic stem cells, spleen cells, pancreas cells, and cells of the central nervous system.

25. The method of claim 24 wherein the cell is a liver cell.

26. The method of claim 21, wherein the cell is a human cell.

27. The method of claim 21, wherein said liver-preferred expression control element comprises at least one enhancer selected from the group consisting of the $\alpha 1$ microglobulin/bikunin enhancer, the hepatitis B virus EnhI enhancer, the hepatitis B virus EnhII enhancer, the human albumin E_{1.7} enhancer, and the human albumin E₆ enhancer.

28. The method of claim 21, wherein said liver-preferred expression control element comprises the hepatitis B virus EnhI enhancer given as about nucleotides 419 to 4835 of the nucleotide sequence set forth in SEQ ID NO:1.

29. The method of claim 21, wherein said liver-preferred expression control element comprises at least one promoter selected from the group consisting of the

hepatitis B virus core promoter, the mouse albumin promoter, the human U1 snRNA promoter, the herpes simplex virus thymidine kinase promoter.

30. The method of claim 21, wherein said liver-preferred expression control element comprises at least one transcription factor binding site selected from the group consisting of a TATA box, a CAAT box, a GC box, an ATF box, a C/EBP binding site, an HNF1 binding site, an HNF2 binding site, an HNF3 binding site, an HNF4 binding site, and a TGT3 binding site.

31. The method of claim 21, wherein said tAAV vector additionally comprises at least one AAV ITR operably linked to said nucleotide sequence encoding B-domain deleted factor VIII such that said AAV ITR drives expression of said nucleotide sequence encoding B-domain deleted factor VIII.

32. The method of claim 21, wherein the B-domain deleted factor VIII is a human B-domain factor VIII.

33. The method of claim 32, wherein said heterologous nucleotide sequence encodes a B-domain deleted factor VIII having the amino acid sequence set forth in SEQ ID NO:2.

34. The method of claim 33, wherein said heterologous nucleotide sequence comprises the sequence given as about nucleotides 419 to 4835 of the nucleotide sequence set forth in SEQ ID NO:1.

35. A method of delivering a nucleotide sequence encoding a B-domain deleted factor VIII to a cell comprising contacting the cell with a recombinant adeno-associated virus (rAAV) vector comprising a heterologous nucleotide sequence encoding a B-domain deleted factor VIII selected from the group consisting of:

- (a) the nucleotide sequence given as nucleotides 419 to 4835 of the nucleotide sequence set forth in SEQ ID NO:1,
- (b) a nucleotide sequence that hybridizes to the nucleotide sequence of (a) under conditions of high stringency and which encodes a B-domain deleted factor VIII, and
- (c) a nucleotide sequence that differs from the nucleotide sequences of (a) and (b) above due to the degeneracy of the genetic code, and which encodes a B-domain deleted factor VIII.

36. A method of delivering a nucleotide sequence encoding B-domain deleted factor VIII to a cell comprising contacting the cell with a composition comprising a population of recombinant adeno-associated virus (AAV) vectors comprising a heterologous nucleotide sequence encoding B-domain-deleted factor VIII, and further wherein said composition has a titer of at least about 10^8 infectious units per milliliter.

37. A method of enhancing blood coagulation in a subject in need thereof comprising administering a recombinant adeno-associated virus (rAAV) vector comprising a heterologous nucleotide sequence encoding B-domain deleted factor VIII to the subject in an amount sufficient to enhance blood coagulation.

38. The method of claim 37, wherein at least about 2×10^{10} particles of the rAAV vector are administered to the subject.

39. The method of claim 37, wherein the subject is a mammalian subject.

40. The method of claim 39, wherein the subject is a human subject.

41. The method of claim 40, wherein the rAAV vector is administered by a route selected from the group consisting of oral, rectal, transmucosal, transdermal,

inhalation, intravenous, subcutaneous, intradermal, intracranial, intramuscular, and intraarticular administration.

42. The method of claim 41, wherein the rAAV is administered to the liver of the subject.

43. The method of claim 44, wherein the rAAV is administered to the liver by a route selected from the group consisting of intravenous administration, intraportal administration, intrabiliary administration, intra-arterial administration, and direct injection into the liver parenchyma.

44. The method of claim 37, wherein the rAAV further comprises a liver-preferred expression control element operably linked with the heterologous nucleotide sequence encoding factor VIII.

45. The method of claim 44, wherein said liver-preferred expression control element comprises at least one enhancer selected from the group consisting of the $\alpha 1$ microglobulin/bikunin enhancer, the hepatitis B virus EnhI enhancer, the hepatitis B virus EnhII enhancer, the human albumin E_{1.7} enhancer, and the human albumin E₆ enhancer.

46. The method of claim 45, wherein the liver-preferred expression control element is a hepatitis B virus enhancer element EnhI or a hepatitis B virus enhancer element EnhII.

47. The method of claim 37, wherein the B-domain deleted factor VIII is a human B-domain deleted factor VIII.

48. The method of claim 47, wherein the heterologous nucleotide sequence encodes a B-domain deleted factor VIII having the sequence given in SEQ ID NO:2.

49. The method of claim 48, wherein the heterologous nucleotide sequence encodes the amino acid sequence set forth in SEQ ID NO:2.

50. A method of treating hemophilia A comprising administering to a hemophiliac subject a biologically effective amount of a recombinant adeno-associated virus (rAAV) vector comprising a heterologous nucleotide sequence encoding B-domain deleted factor VIII, wherein said B-domain deleted factor VIII is expressed at therapeutically effective amounts.

51. A method of treating hemophilia comprising administering to the liver of a hemophiliac subject, a biologically effective amount of a recombinant adeno-associated virus (rAAV) vector comprising a heterologous nucleotide sequence encoding B-domain deleted factor VIII.

52. The method of claim 51, wherein the liver expresses the encoded B-domain deleted factor VIII, which is secreted into the blood in a therapeutically effective amount.

53. A method of administering B-domain deleted factor VIII to a subject comprising administering a cell expressing B-domain deleted factor VIII to the subject, wherein the cell has been produced by a method comprising contacting the cell with a recombinant adeno-associated virus (rAAV) vector comprising a nucleotide sequence encoding B-domain deleted factor VIII.

54. The method of claim 53, wherein the cell is selected from the group consisting of hematopoietic stem cells, liver cells, fibroblasts, epithelial cells, spleen cells, pancreatic cells, keratinocytes, endothelial cells, myoblasts, and neural cells.

55. A method of producing a high-titer stock of a recombinant adeno-associated virus (rAAV) vector comprising

- 5
- (a) infecting a packaging cell with a rAAV vector comprising a heterologous nucleotide sequence encoding factor VIII,
 - (b) allowing the rAAV genome to replicate and be encapsidated by the packaging cell, and
 - (c) collecting the rAAV particles to form a rAAV stock;
- wherein the titer of the rAAV stock is at least about 10^6 infectious units per milliliter.

10 56. The method of claim 55, wherein the heterologous nucleotide sequence encoding factor VIII is operably linked with a liver-preferred expression control element.

57. A virus stock produced by the method of claim 55.

15 *Page 5* 58. A nucleotide sequence encoding B-domain deleted factor VIII operably linked with a hepatitis virus expression control element.

59. The nucleotide sequence of claim 58, wherein said hepatitis virus expression control element is from a hepatitis B virus.

20 60. The nucleotide sequence of claim 59, wherein said hepatitis virus expression control element is a hepatitis B virus EnhI or EnhII enhancer.

61. The nucleotide sequence of claim 60, wherein said hepatitis virus expression control element is a hepatitis B virus EnhI enhancer.

25 62. The nucleotide sequence of claim 58, wherein said nucleotide sequence comprises the sequence given as about nucleotides 150 to 4835 of the nucleotide sequence set forth in SEQ ID NO:1.

63. The nucleotide sequence of claim 62, wherein said nucleotide sequence further comprises a promoter and a polyadenylation sequence.

5 64. The nucleotide sequence of claim 63, wherein said nucleotide sequence comprises the sequence given as nucleotides 150 to 4914 of the nucleotide sequence set forth in SEQ ID NO:1.

65. A vector comprising the nucleotide sequence of claim 58.

10 66. The vector of claim 65, wherein said vector is the plasmid disclosed herein as pDLZ6.

15 67. A cell containing the vector of claim 65.

add A2 *add B15*